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Abbreviations

AhR	aryl hydrocarbon receptor
BMI	body mass index
CI	confidence interval
HbA1c	glycated hemoglobin
HDL-C	high density lipoprotein cholesterol
HR	hazard ratio
MET	metabolic equivalent of task
OR	odds ratio
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzodioxins
PCDFs	polychlorinated dibenzofurans
PPAR	peroxisome proliferator-activated receptor
ppt	parts per trillion
SWHS	Seveso Women's Health Study
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TEQ	toxic equivalent

ABSTRACT

Background: In animal studies, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters glucose transport and increases lipids and blood pressure. Epidemiologic evidence suggests an association between TCDD and metabolic disease.

Objectives: On July 10, 1976, a chemical explosion in Seveso, Italy, resulted in the highest known residential exposure to TCDD. Using data from the Seveso Women's Health Study (SWHS), a cohort study of the health of the women, we examined the relation of serum TCDD to diabetes, metabolic syndrome, and obesity over 30 years later.

Methods: In 1996, we enrolled 981 women who were newborn to 40 years in 1976 and resided in the most contaminated areas. Individual TCDD concentration was measured in archived serum collected soon after the explosion. In 2008, 833 women participated in a follow-up study. Diabetes was classified based on self-report or fasting serum glucose and glycated hemoglobin levels. Metabolic syndrome was defined by International Diabetes Federation criteria. Obesity was defined as body mass index $\geq$30 kg/m^2.

Results: A 10-fold increase in serum TCDD (log$_{10}$TCDD) was not associated with diabetes (adjusted hazard ratio = 0.76; 95% confidence interval (CI): 0.45, 1.28) or obesity (adjusted odds ratio = 0.80; 95% CI: 0.58, 1.10). Log$_{10}$TCDD was associated with metabolic syndrome, but only among women who were $\leq$ 12 years at explosion (adjusted odds ratio = 2.03; 95% CI: 1.25, 3.29; *p*-interaction = 0.01).

Conclusions: We found an increase in metabolic syndrome associated with TCDD, but only among women who were youngest at exposure. Continued follow-up of the SWHS cohort will be informative.

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a widespread environmental contaminant and potent endocrine disruptor (Birnbaum and Tuomisto 2000; Zook and Rappe 1994). Endocrine-disrupting compounds are hypothesized to have a direct or indirect role in the pathogenesis of metabolic disorders including obesity, metabolic syndrome, and diabetes (Casals-Casas and Desvergne 2011; Hectors et al. 2011; Swedenborg et al. 2009). Experimental studies support a link between TCDD exposure and metabolic alterations. TCDD exposure has been associated with wasting syndrome in rodents fed a normal diet (Seefeld et al. 1984), and with accelerated weight gain in mice fed a high-fat diet (Zhu et al. 2008). Effects on glucose homeostasis have also been reported based on *in vivo* and *in vitro* studies, including reduced glucose uptake in adipose tissue, liver, and pancreas; altered glucose tolerance; and impaired insulin secretion (Enan et al. 1992a, b; Enan and Matsumura 1993; Ishida et al. 2005; Kurita et al. 2009). In mice, TCDD exposure was shown to be associated with increased serum triglycerides, cholesterol, and blood pressure, and with earlier onset and greater severity of atherosclerosis (Dalton et al. 2001).

Several epidemiologic studies have examined the relation of exposure to TCDD and other dioxin-like compounds with diabetes, but most are cross-sectional in design and unable to establish temporal relationships between exposures and outcomes. Results from the few longitudinal studies that have been conducted are inconsistent. An increased risk for diabetes has been reported in Vietnam veterans (Kang et al. 2006; Michalek and Pavuk 2008), phenoxyherbicide production workers (Vena et al. 1998), and Yucheng accident cohort members (Wang et al. 2008), but not in Great Lakes sport fish consumers (Turyk et al. 2009), Finnish fishermen and their wives (Turunen et al. 2008), young US adults (Lee et al. 2010), or elderly Swedish adults (Lee et al. 2011a). Higher risks among women than men were reported in the

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Yucheng cohort (Wang et al. 2008), but few studies have had sufficient power to examine the risk of diabetes in women separately. Recent cross-sectional studies also suggest a positive association of dioxin-like compounds with metabolic syndrome and its individual components (Chang et al. 2010a; Chang et al. 2010b; Lee et al. 2007; Uemura et al. 2009).

On July 10, 1976, a chemical explosion in Seveso, Italy resulted in the highest known residential exposure to TCDD (Mocarelli et al. 1988). Up to 30 kilograms of TCDD were deposited over the surrounding 18 km^2-area (di Domenico et al. 1980), which was divided into exposure zones (A, B, R, non-ABR) based on surface soil TCDD measurements. An ecologic study reported excess mortality from diabetes (1976-2001) among female residents of Zone B, the second most contaminated area (n=20 deaths, relative risk = 1.78 (95% confidence interval (CI): 1.14, 2.77)), but not among males (Consonni et al. 2008). However, diabetes was likely underreported as a cause of death, and no individual-level TCDD exposure data were available.

The Seveso Women's Health Study (SWHS), initiated in 1996, is a historical cohort study of the female population residing around Seveso at the time of the explosion in 1976. SWHS represents the largest female population with known individual-level TCDD exposure, measured in archived serum collected soon after the explosion (Eskenazi et al. 2000). Here, we examine the relation of serum TCDD levels in 1976 with metabolic disorders including diabetes, metabolic syndrome, and obesity in SWHS, over 30 years later.

METHODS

Study Population

Details of the SWHS study design are presented elsewhere (Eskenazi et al. 2000). Briefly, eligible women were newborn to age 40 years in 1976, resided in the most highly contaminated areas at the time of explosion, and had adequate stored sera collected soon after the explosion. Enrollment took place from March 1996 to July 1998 and 981 women (80% of those eligible) participated. Between April 2008 and December 2009, we conducted a follow-up of the SWHS cohort; 16 (1.6%) of the women were deceased, 36 (3.7%) could not be located, and of the women who could be contacted, 833 (85%) participated.

For the diabetes analysis, we included the full SWHS cohort (n=981). For the obesity analysis, we included all women who participated in the 2008 follow-up study (n=833). For the metabolic syndrome analysis, we excluded 27 women who did not undergo a fasting blood draw in 2008 (n=806).

Procedure

The study was approved by the Institutional Review Boards of the participating institutions. Details of the study procedure for the initial study (1996-1998) are presented elsewhere (Eskenazi et al. 2000). Briefly, participation included signed informed consent, fasting blood draw, anthropometric measurements, personal interview, medical records, and for most women, a gynecologic examination and ultrasound. Details of the follow-up study (2008-2009) are also presented elsewhere (Warner et al. 2011). Participation included signed informed consent, fasting blood draw, anthropometric and blood pressure measurements, a personal interview

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including the European Prospective Investigation into Cancer and Nutrition-Italy food frequency questionnaire (Pisani et al. 1997), a memory test, and for a subset, a bone density examination. Additional data were abstracted from medical records. The food frequency questionnaire was analyzed to estimate levels of nutrient components (Pala et al. 2003).

For both the initial and follow-up study, interviews were conducted in private by trained nurse-interviewers who were unaware of the zone of residence and serum TCDD levels of the participants. During the interviews, a detailed reproductive and medical history was recorded and information on demographic and lifestyle factors was collected. Reproductive information included reproductive diseases, age at menarche, pregnancy history, menopause status, and history of hormone use. Current information on other risk factors included use of cigarettes, alcohol or caffeine, physical activity (occupational, recreational, household), and social class factors (education, occupation, income). The medical history included a series of questions about diabetes, medications use, and family history of diabetes.

Anthropometric measurements including height (cm), weight (kg), and waist circumference (cm) were measured at each study; duplicate measures were made and averaged for analysis. We calculated body mass index (BMI, kg/m^2) and classified women as "overweight" or "obese" if they had a BMI ≥ 25 and <30 kg/m^2, or ≥ 30 kg/m^2, respectively (World Health Organization 1998).

At the 2008 follow-up study, resting blood pressure was measured at three 1-minute intervals; and the values were averaged for analysis. Glucose, glycated hemoglobin (HbA1c), triglycerides, and high-density lipoprotein cholesterol (HDL-C) were measured in fasting blood. Metabolic syndrome cases were diagnosed based on the presence of three or more of the following five

criteria: 1) waist circumference ≥ 88 cm; 2) serum triglycerides ≥ 150 mg/dL; 3) serum HDL-C < 50 mg/dL; 4) systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or current use of antihypertensive medication; 5) serum glucose ≥ 100 mg/dL or report of current use of diabetes medication (Alberti et al. 2009).

Diabetes cases were defined by self-report of diagnosis after July 10, 1976 or by fasting serum glucose and HbA1c levels at the most recent examination. During the interview each woman was asked, "Has a doctor ever told you that you had diabetes?" If she answered "yes", she was asked a series of questions about her age at first diagnosis, if she was first diagnosed during pregnancy, and about her treatment history. If a woman was first diagnosed during pregnancy, but the condition continued after the pregnancy ended, she was considered a diabetes case. If a woman reported she was first diagnosed during pregnancy but had diabetes only during gestation, she was not considered a diabetes case. In addition, women with a current serum glucose ≥ 126 mg/dL or HbA1c ≥ 6.5% were classified as undiagnosed diabetes cases (American Diabetes Association 2011).

Laboratory Analyses

TCDD was measured in archived sera by high-resolution gas chromatography/ high-resolution mass spectrometry methods (Patterson et al. 1987). Details of serum sample selection are presented elsewhere (Eskenazi et al. 2000; Eskenazi et al. 2004). Values are reported on a lipid-weight basis as pg/g lipid or parts-per-trillion (ppt) (Akins et al. 1989). For non-detectable values (n=96), a serum TCDD level of one-half the detection limit was assigned (Hornung and Reed 1990). For the study median serum sample weight of 0.65 g, the median limit of detection was 18.8 ppt, lipid-adjusted.

Statistical Analyses

Because the serum TCDD distribution was approximately log-normal, TCDD levels were $\log_{10}$-transformed. Serum TCDD was analyzed both as a continuous variable ($\log_{10}$TCDD) and a four-category variable. The cut-point for the lowest category was set at ≤20 ppt, because 15-20 ppt was the average TCDD level in serum pools collected from unexposed Italian women in 1976 (Eskenazi et al. 2004). The three remaining categories were defined by calculating tertiles of exposure >20 ppt, producing groups ≤20, 20.1-47.0, 47.1-135.0 and >135 ppt.

We used Cox proportional hazards regression models to examine the relation of serum TCDD to occurrence of diabetes. Age was the underlying time variable, with entry defined as the woman's age on July 10, 1976, and exit defined as her age at diabetes diagnosis (age at diagnosis for self-reported cases, age at blood draw for undiagnosed cases) or censoring (age at death, age at 2008 follow-up study for women who participated in 2008, age at 1996 study for women who did not participate in 2008). One woman reported a diabetes diagnosis before the date of the explosion, and therefore did not enter the Cox models. We used logistic regression to examine the relation of serum TCDD with metabolic syndrome and its individual criteria. We used linear regression to examine the relation of serum TCDD with BMI and polytomous logistic regression to examine the relation of serum TCDD with odds of overweight (BMI ≥ 25 kg/m² and < 30 kg/m²) and obesity (BMI ≥ 30 kg/m²) as separate outcomes, with underweight/normal (BMI < 25 kg/m²) as the reference outcome.

We evaluated a broad range of potential confounding factors identified in the diabetes and metabolic syndrome literature, some of which are listed in Table 1. Covariate information for each woman was based on data collected at last interview. Covariates were included in the final

multivariate models if they changed the coefficient for $\log_{10}$TCDD by more than 10% or if they were independently associated with the outcome ($p<0.10$). For all outcomes, we considered effect modification by developmental status at exposure including: menarche status at explosion (pre-menarche vs post-menarche) and age at explosion (≤ 12 vs >12 years). For diabetes and metabolic syndrome, we additionally considered effect modification by obesity (<30 vs ≥ 30 kg/m^2) status. Effect modification was modeled by creating a product term between $\log_{10}$TCDD and the effect modifier of interest. Interactions were considered significant if the p-value for the product term was <0.2.

In sensitivity analyses, we evaluated effect modification by age using alternative age at explosion cut points ranging from 5 to 13 years. We repeated the adjusted models for diabetes excluding three women with possible type I diabetes based on reported age at diagnosis and a history of insulin use. We also repeated the final models for diabetes, stratifying on diagnosis status (self-report (n=40) versus undiagnosed cases (n=14)). We repeated models of the association between exposure and metabolic syndrome after excluding cases with diabetes (n=40).

For all regression models, standard errors were estimated using the robust Huber-White sandwich estimator. In Cox models, the proportional hazards assumption was tested using scaled Schoenfeld residuals. We conducted tests for linear trend by including categorical TCDD as a continuous term (coded as 0, 1, 2, 3) in the models. All statistical analyses were performed using STATA 11.0 (Stata Corp 2009).

RESULTS

Demographic characteristics of the 981 women in the SWHS cohort are presented in Table 1. On the date of the explosion, the average age of the full cohort was 20 years, 29% were premenarcheal and 0.5% were postmenopausal. At last interview, women averaged 50.9 years and about half (52%) were postmenopausal. The majority of women (63%) had never regularly smoked or consumed alcohol, 12.7% reported no leisure-time physical activity (0 MET-hours/day) and 22% were obese. About 29% of women reported a family history of diabetes in a parent or sibling. Characteristics of the 833 women who participated in the 2008 follow-up study are similar to those of the full cohort (see Table 1).

The median lipid-adjusted TCDD concentration measured in blood collected near the time of the explosion in 1976 for the full cohort (n=981) was 55.9 ppt (interquartile range = 28, 157). Median serum TCDD levels were higher among women who were youngest at the time of the explosion (165.0 ppt for women age 0-10 years versus 45.6 ppt for women age 11-40 years) or who were still premenarche at explosion (142.5 ppt for premenarche versus 44.4 ppt for postmenarche), as reported previously (Eskenazi et al. 2004). Median serum TCDD levels were also higher in women who at last interview were never married, more educated, currently employed, or premenopausal, but these likely reflect the higher TCDD levels in younger women (data not shown).

Over the 32-year follow-up period, 54 women (5.5%) in the cohort were diagnosed with diabetes. Of the 54 cases, 40 were identified by self-report of a doctor's diagnosis and an additional 14 were diagnosed by fasting serum glucose or Hb1Ac levels at the most recent blood draw. The average age at diagnosis of all 54 cases was 54.9 (±9.4) years. The geometric mean

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(geometric standard deviation) serum TCDD level for the diabetes cases [47.9 (3.7) ppt] was significantly less than for the non-cases [n=926, 71.1 (4.2) ppt] (ANOVA for $\log_{10}$TCDD: $p = 0.05$). As presented in Table 2, the adjusted HR for diabetes associated with a 10-fold increase in TCDD ($\log_{10}$TCDD) was 0.76 (95% CI: 0.45, 1.28). When TCDD was modeled as a categorical variable, there was some evidence of an inverse dose-response trend ($p=0.09$). The trend was not monotonic, as the hazard of diabetes was non-significantly increased in the second TCDD quartile, but was non-significantly decreased in the third and fourth quartiles. We found no evidence of effect modification by obesity status (for non-obese, adjusted HR = 0.68; 95%CI: 0.32, 1.44; for obese, adjusted HR = 0.85; 95% CI: 0.41, 1.78; p-interaction=0.68). There were too few cases to examine potential effect modification by menarche status (n=2 cases premenarche) or age at explosion (n=3 cases $\leq$ 12 years).

The average BMI of the 833 women who participated in the 2008 follow-up study was 26.7 ($\pm$ 5.5) kg/m^2; 263 (32%) women were overweight and 200 (24%) were obese. We excluded one woman with a BMI of 57.5 kg/m^2 from the linear regression models of BMI due to excessive influence on the results. As presented in Table 3, $\log_{10}$TCDD was not associated with BMI (adjusted β = -0.31; 95% CI: -0.88, 0.27). When BMI was categorized, compared to normal weight (BMI<25 kg/m^2), there was a non-significant reduction in odds of overweight (adjusted OR= 0.85; 95% CI: 0.64, 1.13) and obesity (adjusted OR = 0.80; 95% CI: 0.58, 1.10) per 10-fold increase in serum TCDD. When serum TCDD was categorized, a significant inverse non-monotonic trend was observed for BMI, overweight, and obesity (p-trend = 0.05, 0.05, and 0.04, respectively). We found no evidence of effect modification by menarche status at explosion (p-interaction = 0.94, 0.52, and 0.94) or age at explosion ($\leq$12 vs >12 years) (p-interaction =0.45, 0.65, and 0.38) for BMI, overweight, and obesity, respectively. However, results tended to be

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further from the null in women >12 years compared with women ≤12 years at explosion (for

example, for BMI, adjusted β = -0.02; 95% CI: -0.84, 0.80 for women ≤12 years and adjusted β

= -0.46; 95% CI: -1.25, 0.33 for women >12 years; p-interaction=0.45).

In total, 172 (21%) of the 806 women who underwent a fasting blood draw in 2008 met the

diagnostic criteria for metabolic syndrome. Of these, about half (52.9%) of the cases met three

criteria for diagnosis, 30.8% met four criteria, and 16.3% met all five criteria. The most prevalent

criteria were high blood pressure (47.5%) and increased waist circumference (39.1%). As

presented in Table 4, the overall adjusted OR for metabolic syndrome with a 10-fold increase in

TCDD was close to 1.0 (adjusted OR=1.05; 95% CI: 0.78, 1.43), but there was evidence of effect

modification by age at explosion (p=0.01). There was a significant positive association of

metabolic syndrome with log_{10}TCDD among women who were age 12 years or less at the time

of the explosion (adjusted OR = 2.03; 95% CI: 1.25, 3.30), but no association among women

who were more than 12 years at the time of the explosion (adjusted OR = 0.96; 95% CI: 0.68,

1.35). A similar difference between the older and younger groups was noted for some of the

individual criteria. For example, there was effect modification by age at explosion for high blood

pressure (for ≤ 12 years, adjusted OR = 1.45; 95% CI: 0.95, 2.21; for > 12 years, adjusted OR =

0.54; 95% CI: 0.38, 0.77; p-interaction < 0.01). However, there was little evidence of effect

modification by age at explosion for the HDL-C, triglycerides, or glucose indicators.

We found no evidence of effect modification by menarche status at explosion (p=0.57). In

sensitivity analyses, we considered effect modification by other age at explosion cut points,

although at younger ages the number of cases is small. For 6 to 13 years, the interaction p-value

was < 0.20, with an increased risk of metabolic syndrome in the younger group (significant at p

< 0.05 for ages 11 and 12, and at p < 0.10 for ages 9, 10, and 13). ORs in the younger group

ranged from 1.60 to 2.03, and ORs in the older group were close to 1.00 at all ages. Results were strongest and most significant for the 12-year age at explosion cut point.

In sensitivity analyses, we repeated the final models for diabetes excluding 3 possible type I diabetes cases (age of onset: 25, 34, and 37 years) and stratifying on diagnosis status (self-report (n=40) versus undiagnosed (n=14)) and the results were similar (data not shown). We repeated the final models for metabolic syndrome excluding diabetes cases (n=40) and the results were similar (data not shown).

DISCUSSION

To our knowledge, this is the first epidemiologic study to prospectively examine the relation of individual serum TCDD levels and metabolic disorders including diabetes, metabolic syndrome, and obesity in a highly exposed population. In this study of women residing in Seveso, Italy, in 1976, at the time of an explosion that resulted in very high levels of TCDD exposure, we found a significant positive association between metabolic syndrome and serum TCDD levels, but only among women who were youngest at exposure. In contrast, obesity was not associated with TCDD, and this association did not vary with age at exposure. Finally, we found no association of serum TCDD levels with diabetes, but there were too few cases to examine possible effect modification by age at exposure.

The observed association between TCDD in 1976 and metabolic syndrome in 2008 is consistent with recent cross-sectional studies suggesting a positive association between TCDD and dioxin-like compounds with prevalence of metabolic syndrome (Chang et al. 2010a; Chang et al. 2010b; Uemura et al. 2009). A significant increasing trend in prevalence of metabolic syndrome was

reported with increasing quintiles of TCDD and total toxic equivalents (TEQ) (based on polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs)) measured in serum of 1,490 Taiwanese residents living in the vicinity of an abandoned pentachorophenol factory (Chang et al. 2010b). The adjusted ORs for the highest quintiles of TCDD and total TEQ were 2.8 (95% CI: 1.6, 4.9) and 2.3 (95% CI: 1.3, 3.9), respectively. In a population-based study of 1,374 Japanese residents, a significant increasing trend in prevalence of metabolic syndrome was reported with increasing quartiles of total TEQ (based on PCDDs, PCDFs, and dioxin-like polychlorinated biphenyls (PCBs)) (Uemura et al. 2009). The adjusted OR in the highest quartile of total TEQ was 5.3 (95% CI: 2.3, 13.0). Our results are also consistent with the hypothesis that early life exposure to endocrine disrupting compounds, such as TCDD, increases risk of metabolic disorders later in life. We observed a relationship between TCDD and metabolic syndrome that was limited to women who were 12 years or less at exposure. Although median serum TCDD levels are higher in the youngest women, there was a wide range of exposure in both groups ($\leq$12 years: 3.4-56,000 ppt, >12 years: 2.5-6,320 ppt). This range allowed us to estimate the relationship between TCDD and metabolic syndrome in both age groups separately. It is noteworthy that the prevalence of metabolic syndrome (6%) observed among the youngest women in this study ($\leq$ 12 years at the time of explosion) is almost double the prevalence (~3.3%) estimated for similar aged women from a nearby region of Italy (Miccoli et al. 2005).

We did not find a significant association of serum TCDD levels with BMI or obesity. However, our finding of a significant non-monotonic decreasing trend in BMI associated with higher serum TCDD levels is somewhat consistent with two recent epidemiologic studies of dioxin-like PCBs. In a small prospective nested case-control study, a significant decreasing trend in BMI was associated with dioxin-like PCB 156 (but not other dioxin-like PCBs 105, 118, 157, 167),

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measured in serum in 90 controls (Lee et al. 2011b). A cross-sectional study of older Swedish residents reported a significant decreasing trend in total body fat mass measured by dual x-ray absorptiometry with increasing quartiles of serum dioxin-like PCBs (126, 169, 156, 157, 189) (Ronn et al. 2011). The results of these studies and the present one demonstrating a negative association between TCDD and BMI are not necessarily contradictory to our observed association of TCDD with waist circumference ≥ 88 cm among those ≤ 12 years at exposure (adj-OR = 1.32; 95% CI: 0.84, 2.06), as waist circumference is considered a better marker of central adiposity and "metabolic unfitness" than BMI (Alberti et al. 2009). Since central adiposity is a strong risk factor for cardiovascular disease, it is plausible that we would find a relationship with waist circumference and not BMI despite their high correlation (r=0.88).

Our finding of no association of serum TCDD levels with diabetes is not consistent with previous prospective studies that reported a positive association between TCDD exposure and diabetes (Consonni et al. 2008; Kang et al. 2006; Michalek and Pavuk 2008; Vena et al. 1998; Wang et al. 2008) nor with previous reports of stronger associations among women than men (Wang et al. 2008). However, there are a number of possible explanations for the difference in findings. Some studies examined risk of diabetes mortality (Consonni et al. 2008; Vena et al. 1998). There was a wide variation in classification of exposure across studies. Some measured TCDD exposure in serum collected years after last exposure (Kang et al. 2006; Michalek and Pavuk 2008), others measured contemporary levels of some but not all dioxin-like PCB compounds in serum (Lee et al. 2010; Lee et al. 2011a; Turyk et al. 2009), while others reported an ecologic measure such as job category (Kang et al. 2006; Vena et al. 1998; Wang et al. 2008). However, given the association between TCDD and metabolic syndrome observed in the

youngest age group, a longer follow-up period is needed in this susceptible group to determine if these younger women develop diabetes as they age.

There is strong biologic plausibility for the metabolic disruption effects of TCDD. Most effects of TCDD are mediated through the aryl hydrocarbon receptor (AhR) (Hankinson 1995; Okey et al. 1994), and the AhR has been implicated as a regulator of energy metabolism. TCDD alters glucose and lipid metabolism in mice (Dalton et al. 2001), modifies expression of genes related to insulin transport and signaling pathways in human adipose tissue (Fujiyoshi et al. 2006), and produces oxidative stress at high concentrations (Kern et al. 2002; Matsumura 2003). In C57BL/6N mice, TCDD has been shown to affect expression of genes related to hepatic circadian rhythm, cholesterol biosynthesis, fatty acid synthesis, glucose metabolism, and adipocyte differentiation in an AhR-dependent manner (Arsenescu et al. 2008; Sato et al. 2008). It has been hypothesized that TCDD may affect energy metabolism by altering mRNA expression of corticotropin-releasing factor in the central nervous system or by affecting levels of adiposity signals including insulin and leptin (Linden et al. 2010). In addition, several direct and indirect mechanisms including cross-talk with the estrogen receptor have been proposed (Casals-Casas and Desvergne 2011; Pascussi et al. 2008). A link between TCDD and diabetes has also been hypothesized through interaction between the AhR and peroxisome proliferator–activated receptor (PPAR) γ-mediated signaling pathways (Remillard and Bunce 2002). The PPARγ is a ligand-activated transcription factor involved in lipid metabolism and homeostasis that has been identified as the molecular target of insulin-sensitizing agents used to treat type II diabetes (Smith 2002).

This study has several strengths including the prospective design and ability to measure multiple outcomes related to metabolic disruption by TCDD. We were able to measure individual serum

TCDD concentrations near the time of exposure and preceding the diagnosis of metabolic disease. This study represents the largest female population with known individual-level TCDD exposure. An advantage over the previous ecologic study in the Seveso population is we were able to measure diabetes occurrence, not mortality, thus eliminating potential biases associated with variations in disease survival. In addition, we were able to collect information on confounding factors during the interview that were not available in other studies.

A limitation of this study is that diabetes cases were diagnosed by self-report; however, we were able to include previously "undiagnosed" cases using standardized criteria for fasting glucose and HbA1c measurements. The small number of diabetes cases in the youngest age group prevented us from examining possible effect modification by age at explosion or menarche status. With aging of the SWHS cohort and continued follow-up, it should be possible to examine evidence of interaction by developmental status at exposure.

In summary, this is the first prospective study to examine the relation of individual serum TCDD levels and metabolic disorders in a highly exposed female population. We estimated a significant positive association between serum TCDD level in 1976 and metabolic syndrome in 2008 among women who were ≤12 years of age at exposure. In contrast, obesity was not associated with TCDD levels, regardless of age at exposure. We found no evidence of increased risk for diabetes; however, we were unable to examine effect modification by age at exposure. These results are generally consistent with effects of TCDD noted in animal studies and with greater sensitivity to TCDD during development, continued follow-up as the SWHS cohort ages will be informative.

REFERENCES

Akins JR, Waldrep K, Bernert JT, Jr. 1989. The estimation of total serum lipids by a completely enzymatic 'summation' method. Clin Chim Acta 184:219-226.

Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. 2009. Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 120:1640-1645.

American Diabetes Association. 2011. Standards of medical care in diabetes--2011. Diabetes Care 34 Suppl 1:S11-61.

Arsenescu V, Arsenescu RI, King V, Swanson H, Cassis LA. 2008. Polychlorinated biphenyl-77 induces adipocyte differentiation and proinflammatory adipokines and promotes obesity and atherosclerosis. Environ Health Perspect 116:761-768.

Birnbaum LS, Tuomisto J. 2000. Non-carcinogenic effects of TCDD in animals. Food Addit Contam 17:275-288.

Casals-Casas C, Desvergne B. 2011. Endocrine disruptors: from endocrine to metabolic disruption. Annu Rev Physiol 73:135-162.

Chang JW, Chen HL, Su HJ, Liao PC, Guo HR, Lee CC. 2010a. Dioxin exposure and insulin resistance in Taiwanese living near a highly contaminated area. Epidemiology 21:56-61.

Chang JW, Ou HY, Chen HL, Guo HR, Liao PC, Lee CC. 2010b. Interrelationship between exposure to PCDD/Fs and hypertension in metabolic syndrome in Taiwanese living near a highly contaminated area. Chemosphere 81:1027-1032.

Consonni D, Pesatori AC, Zocchetti C, Sindaco R, D'Oro LC, Rubagotti M, et al. 2008. Mortality in a population exposed to dioxin after the Seveso, Italy, accident in 1976: 25 years of follow-up. Am J Epidemiol 167:847-858.

Dalton TP, Kerzee JK, Wang B, Miller M, Dieter MZ, Lorenz JN, et al. 2001. Dioxin exposure is an environmental risk factor for ischemic heart disease. Cardiovasc Toxicol 1:285-298.

di Domenico A, Silano V, Viviano G, Zapponi G. 1980. Accidental release of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at Seveso, Italy. II. TCDD distribution in the soil surface layer. Ecotoxicol Environ Saf 4:298-320.

Enan E, Liu PC, Matsumura F. 1992a. 2,3,7,8-tetrachlorodibenzo-p-dioxin causes reduction of glucose transporting activities in the plasma membranes of adipose tissue and pancreas from the guinea pig. J Biol Chem 267:19785-19791.

Enan E, Liu PC, Matsumura F. 1992b. TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) causes reduction in glucose uptake through glucose transporters on the plasma membrane of the guinea pig adipocyte. J Environ Sci Health B 27:495-510.

Enan E, Matsumura F. 1993. 2,3,7,8-tetrachlorodibenzo-p-dioxin induced alterations in protein phosphorylation in guinea pig adipose tissue. J Biochem Toxicol 8:89-99.

Eskenazi B, Mocarelli P, Warner M, Samuels S, Vercellini P, Olive D, et al. 2000. Seveso Women's Health Study: a study of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on reproductive health. Chemosphere 40:1247-1253.

Eskenazi B, Mocarelli P, Warner M, Needham L, Patterson DG, Jr., Samuels S, et al. 2004. Relationship of serum TCDD concentrations and age at exposure of female residents of Seveso, Italy. Environ Health Perspect 112:22-27.

Fujiyoshi PT, Michalek JE, Matsumura F. 2006. Molecular epidemiologic evidence for diabetogenic effects of dioxin exposure in U.S. Air Force veterans of the Vietnam War. Environ Health Perspect 114:1677-1683.

Hankinson O. 1995. The aryl hydrocarbon receptor complex. Annu Rev Pharmacol Toxicol 35:307-340.

Hectors TL, Vanparys C, van der Ven K, Martens GA, Jorens PG, Van Gaal LF, et al. 2011. Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function. Diabetologia 54:1273-1290.

Hornung RW, Reed LD. 1990. Estimation of average concentration in the presence of non-detectable values. Appl Occup Environ Hyg 5:48-51.

Ishida T, Kan-o S, Mutoh J, Takeda S, Ishii Y, Hashiguchi I, et al. 2005. 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced change in intestinal function and pathology: evidence for the involvement of arylhydrocarbon receptor-mediated alteration of glucose transportation. Toxicol Appl Pharmacol 205:89-97.

Kang HK, Dalager NA, Needham LL, Patterson DG, Jr., Lees PS, Yates K, et al. 2006. Health status of Army Chemical Corps Vietnam veterans who sprayed defoliant in Vietnam. Am J Ind Med 49:875-884.

Kern PA, Fishman RB, Song W, Brown AD, Fonseca V. 2002. The effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on oxidative enzymes in adipocytes and liver. Toxicology 171:117-125.

Kurita H, Yoshioka W, Nishimura N, Kubota N, Kadowaki T, Tohyama C. 2009. Aryl hydrocarbon receptor-mediated effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on glucose-stimulated insulin secretion in mice. J Appl Toxicol 29:689-694.

Lee DH, Lee IK, Porta M, Steffes M, Jacobs DR, Jr. 2007. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. Diabetologia 50:1841-1851.

Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs DR, Jr. 2010. Low dose of some persistent organic pollutants predicts type 2 diabetes: a nested case-control study. Environ Health Perspect 118:1235-1242.

Lee DH, Lind PM, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind L. 2011a. Polychlorinated biphenyls and organochlorine pesticides in plasma predict development of type 2 diabetes in the elderly: the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) Study. Diabetes Care 34:1778-1784.

Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs DR, Jr. 2011b. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. PLoS One 6:e15977.

Linden J, Lensu S, Tuomisto J, Pohjanvirta R. 2010. Dioxins, the aryl hydrocarbon receptor and the central regulation of energy balance. Front Neuroendocrinol 31:452-478.

Matsumura F. 2003. On the significance of the role of cellular stress response reactions in the toxic actions of dioxin. Biochem Pharmacol 66:527-540.

Miccoli R, Bianchi C, Odoguardi L, Penno G, Caricato F, Giovannitti MG, et al. 2005. Prevalence of the metabolic syndrome among Italian adults according to ATP III definition. Nutr Metab Cardiovasc Dis 15:250-254.

Michalek JE, Pavuk M. 2008. Diabetes and cancer in veterans of Operation Ranch Hand after adjustment for calendar period, days of spraying, and time spent in Southeast Asia. J Occup Environ Med 50:330-340.

Mocarelli P, Pocchiari F, Nelson N. 1988. Preliminary report: 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure to humans--Seveso, Italy. MMWR Morb Mortal Wkly Rep 37:733-736.

Okey AB, Riddick DS, Harper PA. 1994. The Ah receptor: mediator of the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. Toxicol Lett 70:1-22.

Pala V, Sieri S, Palli D, Salvini S, Berrino F, Bellegotti M, et al. 2003. Diet in the Italian EPIC cohorts: presentation of data and methodological issues. Tumori 89:594-607.

Pascussi JM, Gerbal-Chaloin S, Duret C, Daujat-Chavanieu M, Vilarem MJ, Maurel P. 2008. The tangle of nuclear receptors that controls xenobiotic metabolism and transport: crosstalk and consequences. Annu Rev Pharmacol Toxicol 48:1-32.

Patterson DG, Jr., Hampton L, Lapeza CR, Jr., Belser WT, Green V, Alexander L, et al. 1987. High-resolution gas chromatographic/high-resolution mass spectrometric analysis of human serum on a whole-weight and lipid basis for 2,3,7,8-tetrachlorodibenzo-p-dioxin. Anal Chem 59:2000-2005.

Pisani P, Faggiano F, Krogh V, Palli D, Vineis P, Berrino F. 1997. Relative validity and reproducibility of a food frequency dietary questionnaire for use in the Italian EPIC centres. Int J Epidemiol 26 Suppl 1:S152-160.

Remillard RB, Bunce NJ. 2002. Linking dioxins to diabetes: epidemiology and biologic plausibility. Environ Health Perspect 110:853-858.

Ronn M, Lind L, van Bavel B, Salihovic S, Michaelsson K, Lind PM. 2011. Circulating levels of persistent organic pollutants associate in divergent ways to fat mass measured by DXA in humans. Chemosphere 85:335-343.

Sato S, Shirakawa H, Tomita S, Ohsaki Y, Haketa K, Tooi O, et al. 2008. Low-dose dioxins alter gene expression related to cholesterol biosynthesis, lipogenesis, and glucose metabolism through the aryl hydrocarbon receptor-mediated pathway in mouse liver. Toxicol Appl Pharmacol 229:10-19.

Seefeld MD, Keesey RE, Peterson RE. 1984. Body weight regulation in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 76:526-536.

Smith SA. 2002. Peroxisome proliferator-activated receptors and the regulation of mammalian lipid metabolism. Biochem Soc Trans 30:1086-1090.

Stata Corp. 2009. Stata statistical software: Release 11.0. College Station, TX:Stata Press.

Swedenborg E, Ruegg J, Makela S, Pongratz I. 2009. Endocrine disruptive chemicals: mechanisms of action and involvement in metabolic disorders. J Mol Endocrinol 43:1-10.

Turunen AW, Verkasalo PK, Kiviranta H, Pukkala E, Jula A, Mannisto S, et al. 2008. Mortality in a cohort with high fish consumption. Int J Epidemiol 37:1008-1017.

Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. 2009. Organochlorine exposure and incidence of diabetes in a cohort of Great Lakes sport fish consumers. Environ Health Perspect 117:1076-1082.

Uemura H, Arisawa K, Hiyoshi M, Kitayama A, Takami H, Sawachika F, et al. 2009. Prevalence of metabolic syndrome associated with body burden levels of dioxin and related compounds among Japan's general population. Environ Health Perspect 117:568-573.

Vena J, Boffetta P, Becher H, Benn T, Bueno-de-Mesquita HB, Coggon D, et al. 1998. Exposure to dioxin and nonneoplastic mortality in the expanded IARC international cohort study of phenoxy herbicide and chlorophenol production workers and sprayers. Environ Health Perspect 106:645-653.

Wang SL, Tsai PC, Yang CY, Leon Guo Y. 2008. Increased risk of diabetes and polychlorinated biphenyls and dioxins: A 24-year follow-up study of the Yucheng cohort. Diabetes Care 31:1574-1579.

Warner M, Mocarelli P, Samuels S, Needham L, Brambilla P, Eskenazi B. 2011. Dioxin exposure and cancer risk in the Seveso Women's Health Study. Environ Health Perspect 119:1700-1705.

World Health Organization. 1998. Obesity: preventing and managing the global epidemic. Report of a WHO consultation on obesity Geneva:World Health Organization.

Zhu BT, Gallo MA, Burger CW, Jr., Meeker RJ, Cai MX, Xu S, et al. 2008. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin administration and high-fat diet on the body weight and hepatic estrogen metabolism in female C3H/HeN mice. Toxicol Appl Pharmacol 226:107-118.

Zook D, Rappe C. 1994. Environmental Sources, Distribution, and Fate. In: Dioxins and Health, (Schecter A, ed). New York:Plenum Press, 79-113.

Table 1. Select characteristics of the full SWHS cohort (n=981) and the subset (n=833) who also participated in the 2008 follow-up study, Seveso Women's Health Study, Italy, 1976-2009.

Characteristic		Full Cohort[a] [n (%)]	Participants in 2008 Study[b] [n (%)]
Total		981 (100.0)	833 (84.9)
Characteristics at Explosion			
Zone of residence			
	A	167 (17.0)	142 (17.0)
	B	814 (83.0)	691 (83.0)
Age (years)			
	0-10	232 (23.7)	200 (24.0)
	11-20	279 (28.4)	252 (30.3)
	21-30	241 (24.6)	206 (24.7)
	31-40	229 (23.3)	175 (21.0)
Menarche status			
	Premenarche	284 (29.0)	247 (29.7)
	Postmenarche	697 (71.0)	586 (70.3)
Characteristics at Last Interview			
Menopause status			
	Premenopause	472 (48.1)	394 (47.3)
	Postmenopause	509 (51.9)	439 (52.7)
Education[c]			
	≤Required	651 (66.4)	550 (66.0)
	Secondary school	288 (29.4)	249 (29.9)
	>Secondary school	42 (4.3)	34 (4.1)
Marital status			
	Never	76 (7.7)	47 (5.6)
	Ever	905 (92.3)	786 (94.4)
Employment status			
	Employed	481 (49.0)	408 (49.0)
	Unemployed	500 (51.0)	425 (51.0)
Cigarette smoking			
	Never	619 (63.1)	525 (63.0)
	Former	194 (19.8)	177 (21.3)
	Current	168 (17.1)	131 (15.7)
Alcohol use			
	Never	618 (63.0)	529 (63.5)
	Former	44 (4.5)	32 (3.8)
	Current	319 (32.5)	272 (32.7)
BMI categories			
	Underweight	26 (2.7)	18 (2.2)
	Normal	437 (44.5)	352 (42.3)
	Overweight	302 (30.8)	263 (31.6)
	Obese	216 (22.0)	200 (24.0)

Characteristic	Full Cohort[a] [n (%)]	Participants in 2008 Study[b] [n (%)]
Leisure time physical activity (MET-hrs/day)[d]		
Q1: ≤2.3	245 (25.7)	137 (17.0)
Q2: >2.3–4.7	266 (27.8)	266 (33.0)
Q3: >4.7–7.3	223 (23.4)	216 (26.8)
Q4: >7.3	221 (23.1)	188 (23.3)
Family history of diabetes		
No	701 (71.5)	564 (67.7)
Yes	280 (28.5)	269 (32.3)

[a] Characteristics at last interview) includes data from 2008 for those who participated in the 2008 follow-up study (n=833) and from 1996 for those who did not (n=148).

[b] Characteristics at last interview for participants in the 2008 study (n=833) includes data from 2008.

[c] ≤Required (≤8 years of school), Secondary school (9-13 years), >Secondary school (>13 years)

[d] Missing data for 26 women (all who participated in the 2008 study).

Table 2. Hazard ratios (HR) from Cox proportional hazards models for association between 1976 serum TCDD levels and diabetes risk, Seveso Women's Health Study, Italy, 1976-2009.

Exposure	Cases/Total	HR (95% CI)	Adjusted HR[a] (95% CI)
$Log_{10}TCDD$[b]	54/980	0.85 (0.50, 1.42)	0.76 (0.45, 1.28)
		p=0.53	*p*=0.30
TCDD (ppt)			
≤20	8/154	1.00	1.00
20.1-47.0	27/275	1.58 (0.71, 3.48)	1.53 (0.70, 3.36)
47.1-135.0	11/278	0.79 (0.32, 1.95)	0.75 (0.32, 1.80)
>135	8/273	0.84 (0.31, 2.24)	0.66 (0.24, 1.85)
		p-trend = 0.20	*p*-trend = 0.09

[a] Adjusted for alcohol consumption, waist circumference, and family history of diabetes.

[b] HR for a 10-fold increase in serum TCDD.

Table 3. Adjusted β coefficient from linear regression and odds ratio (OR) from polytomous logistic regression models for the association between 1976 serum TCDD levels and body mass index, overweight, and obesity, Seveso Women's Health Study, Italy, 1976-2009.

Exposure	Body Mass Index (kg/m^2) n (%)	Adj. β[a] (95% CI)	Overweight vs. Normal weight n (%)	Adj. OR[a] (95% CI)	Obese vs. Normal weight n (%)	Adj. OR[a] (95% CI)
Log$_{10}$TCDD[b]	832 (100.0)	-0.31 (-0.88, 0.27)	263 (31.6)	0.85 (0.64, 1.13)	200 (24.0)	0.80 (0.58, 1.10)
TCDD (ppt)						
≤20	132 (15.9)	0.00	48 (36.1)	1.00	42 (31.6)	1.00
20.1-47.0	234 (28.1)	-0.99 (-2.06, 0.09)	81 (34.6)	0.73 (0.43, 1.23)	64 (27.4)	0.57 (0.32, 1.00)
47.1-135.0	232 (27.9)	-1.45 (-2.53, -0.37)	70 (30.2)	0.58 (0.34, 0.97)	50 (21.6)	0.47 (0.26, 0.84)
>135	234 (28.1)	-1.10 (-2.19, -0.01)	64 (27.4)	0.60 (0.36, 1.01)	44 (18.8)	0.52 (0.29, 0.93)
		p-trend=0.05		p-trend=0.05		p-trend=0.04

[a] Adjusted for age at interview, education, and current alcohol consumption.
[b] Estimate for a 10-fold increase in serum TCDD.

Table 4. Adjusted odds ratios (OR) for the association between 1976 serum TCDD levels and metabolic syndrome and individual criteria[a], stratified by age at explosion, Seveso Women's Health Study, Italy, 1976-2009.

Outcome	All Women (N=806)		Age at Explosion				
			≤ 12 Years (n=268)		> 12 Years (n=538)		
	n (%)	Adj. OR[b] (95% CI)	n	Adj. OR[b] (95% CI)	n	Adj. OR[b] (95% CI)	*p*-int
Metabolic Syndrome[c]	172 (21.3)	1.05 (0.78, 1.43)	16	2.03 (1.25, 3.30)	156	0.96 (0.68, 1.35)	0.01
Waist Circumference[d]	315 (39.1)	0.98 (0.75, 1.26)	58	1.32 (0.84, 2.06)	257	0.83 (0.61, 1.14)	0.10
Triglycerides[d]	101 (12.5)	1.02 (0.71, 1.48)	18	1.31 (0.63, 2.70)	83	0.98 (0.65, 1.49)	0.50
HDL Cholesterol[d]	202 (25.1)	1.07 (0.81, 1.41)	59	1.35 (0.87, 2.09)	143	0.98 (0.68, 1.40)	0.26
Blood Pressure[d]	383 (47.5)	0.71 (0.53, 0.94)	44	1.45 (0.95, 2.21)	339	0.54 (0.38, 0.77)	<0.01
Glucose[d]	155 (19.2)	0.94 (0.69, 1.29)	10	0.63 (0.31, 1.27)	145	1.07 (0.76, 1.50)	0.18

[a] Individual criteria: 1) waist circumference ≥88 cm; 2) serum triglycerides ≥150 mg/dL; 3) serum HDL-C <50 mg/dL; 4) blood pressure ≥130/85 mmHg or current use of antihypertensive medication; 5) serum glucose ≥100 mg/dL or report of current use of diabetes medication

[b] OR for a 10-fold increase in serum TCDD

[c] Adjusted for age at interview, leisure time physical activity, family history of diabetes, and current use of medication that may increase serum glucose.

[d] Adjusted for age at interview.